NPIP Lab Audits

Procedures Used in KY, NC & SC

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NPIP Authorized Laboratory Audits

- Definition of an Authorized Laboratory:
  - An authorized laboratory is a laboratory that meets the requirements of §147.51 and is thus qualified to perform the assays described in part 147 of this subchapter.

NPIP Authorized Laboratory Audits

- §147.51 -- Authorized Laboratory Minimum Requirements (originally proposed in 2006)
  1. *Check test proficiency
  2. *Trained technicians
  3. *Laboratory protocol
  4. State site visit
  5. Service review
  6. *Reporting
  7. Verification

  *Requirements checked during audit

NPIP Authorized Laboratory Audits

- How KY, NC and SC does an audit
- Examples of “dummy” audits

NPIP Authorized Laboratory Audits

- Wide range of Authorized Laboratory capabilities
  - Large State labs with American Association of Veterinary Laboratory Diagnosticians (AAVLD) accreditation
  - Medium-sized State or private labs that perform limited testing
  - Private labs that perform only 1 test
    - e.g. company lab just doing AI AGID

Assays Performed at the Lab

- List the assays

Authorized Laboratory Approved to test for the following NPIP Disease Programs:

- ☐ **Pullorum-Typhoid**
  Test Utilized: ☑ Microagglutination Test ☐ Other:_____
  Comments:_____

- ☐ **Avian Influenza**
  Test Utilized: ☑ AGID ☐ ELISA ☐ ACIA ☑ PCR ☐ Other:_____
  Comments:_____

- ☐ **Mycoplasma Synoviae**
  Test Utilized: ☑ Serum Plate Test ☑ ELISA ☐ HI ☐ PCR ☐ Other:_____
  Comments: reactors are sent to PDRC for HI

- ☐ **Mycoplasma Gallisepticum**
  Test Utilized: ☑ Serum Plate Test ☑ ELISA ☐ HI ☐ PCR ☐ Other:_____
  Comments: reactors are sent to PDRC for HI

- ☐ **Salmonella**
  Test Utilized: ☑ Culture for bird samples ☑ Culture for environmental samples
  ☐ PCR based rapid test ☐ Real-Time PCR ☐ Other:_____
  Comments:_____

- ☐ **Diagnostic Work**
  Test Utilized: ☑ Necropsy ☑ Bacteriology ☐ Virology ☑ Serology
  ☐ Toxicology ☑ Histopathology ☑ Molecular Diagnostics
  ☐ Other: _____
  Comments:_____

(1) Check Test Proficiency

- Authorized laboratories must use a regularly scheduled check test for each assay that it performs. *(2010)*

- NPIP will serve as the lead agency for the coordination of available check tests from NVSL (*2010*).

(Proposed changes not approved yet)

(1) Check Test Proficiency

- Not all NPIP tests have “official” check tests:
  - Official – available from NVSL:
    - AI AGID, AI PCR
    - Salmonella culture
  - Unofficial -- available from PDRC:
    - MG/MS convalescent contact infected chicken sera (to use for plate, ELISA, HI testing)
    - MG/MS PCR Proficiency Panel (new in 2012)
  - No official check test:
    - Pullorum-Typhoid plate test, microagglutination test, tube test (can use positive control sera)
    - AI ELISA
    - AI antigen capture

(1) Check Test Proficiency

- For the audit
  - Laboratory keeps proficiency records together for auditor to review and record:
    - From NVSL: letters of pass or no-pass
    - From PDRC: worksheet on technician results
  - Suggest to document on audit:
    - Disease Program
    - Test performed
    - Date performed
    - Score

(1) Check Test Proficiency

Disease	Test Performed	Date	Score
AI	PCR/NCD	2010	pass
Mycoplasma	ELISA HI	6/17/2010 6/17/2010	100% satisfactory
Salmonella Group D	Culture PCR	2010	100%
AI	AGID	12/2010	100% satisfactory
Salmonella Group D	Culture/PCR	5/5/2011	100%
AIV/NDV	PCR by Dally	7/11/2011	100%
AIV/NDV	PCR by Sally	7/11/2011	100%
AIV/NDV	PCR by Patty	7/11/2011	100%
AIV/NDV	PCR by Matty	7/11/2011	100%
AI	**AGID**	**1/20/2012**	**100% satisfactory**
Mycoplasma	**ELISA** **HI**	**2/16/2012** **2/16/2012**	**100%** **satisfactory**
Salmonella Group D	**Culture/PCR**	**7/18/2012**	**100%**
AIV/NDV	**PCR by Dally**	**4/26/2012**	**100%**
AIV/NDV	**PCR by Sally**	**4/26/2012**	**100%**
AIV/NDV	**PCR by Patty**	**4/26/2012**	**100%**
AIV/NDV	**PCR by Matty**	**4/26/2012**	**100%**

(2) Trained Technicians

- The testing procedures at a laboratory must be run or overseen by a lab technician who has attended and satisfactorily completed Service-approved laboratory workshops for Plan specific diseases within the past 3 (4) years. *(2012)*
  - NPIP Training Workshops for Salmonella, Mycoplasma, Avian Influenza

(2) Trained Technicians

- For the audit:
  - Laboratory keep technicians training certificates together for auditor to review and record:
  - Suggest to document on audit:
    - Technician name
    - Training performed by
    - Dates of training
    - Disease or test training

(2) Trained Technicians

Employee	Training by	Date of Training	Disease or Test
Dr. Sally	USDA/NPIP	3/17/2010	Mycoplasma
Johnny B.	USDA/NPIP	3/17/2010	Mycoplasma
Sally May T.	USDA/NPIP	5/20/2010	Salmonella
Matty S.	USDA/NPIP	9/29/2010	Mycoplasma
Chris T.	USDA/NPIP	9/29/2010	Mycoplasma
Henderson J.	USDA/NPIP	10/27/2010	AI
Bessy M	USDA/NPIP	10/27/2010	AI
Henderson R.	USDA/NPIP	8/4/2011	Mycoplasma
Sally May T	**USDA/NPIP**	**11/10/2011**	**AI**
Johnny B.	**USDA/NPIP**	**11/10/2011**	**AI**
Lisa K.	**USDA/NPIP**	**10/27/2011**	**Salmonella**
Patty. R.	**USDA/NPIP**	**10/27/2011**	**Salmonella**
Sally May	**USDA/NPIP**	**5/24/2012**	**Mycoplasma**
Bessy M.	**USDA/NPIP**	**5/24/2012**	**Mycoplasma**

(3) Laboratory Protocol

- Official Plan assays must be performed and reported as described in Part 147 or the reagent manufacturer (*2010*).

(3) Laboratory Protocol

- Suggestions for this section:
  - Review “Good Laboratory Practices”
    - Standard Operating Procedures (SOP’s)
    - Equipment
    - Accommodation and Environmental Conditions
    - Quality Control
    - Specimens
    - Reporting Test Results

(3) Laboratory Protocol

- Suggestions for this section:
  - Review Standard Operating Procedures (SOP's)
    - Written instructions for all testing activities
    - Properly referenced (e.g. NPIP, NVSL)
    - Document control to ensure current version of SOP is available
      - » Only one version of the SOP is valid – show this through Document Control procedures

(3) Laboratory Protocol

- SOP review -- KY example
- Request all copies of SOP's prior to audit
  - SOP's that had been **revised** since the last NPIP audit were provided in advance of audit.

	SOP	Version Date	Version
S	Avian Influenza AGID	10/25/2011	4.3
S	Avian Influenza ELISA	10/25/2011	1.0
S	Mycoplasma HI	10/25/2011	4.1
M	Gel Electrophoresis Using Gel Logic 212PRO	5/2/2012	1.0
B	WI Environmental Salmonella using MSRV	4/17/2012	1.0

(3) Laboratory Protocol

- SOP review -- KY example
  - The following SOP's and written instructions were on file with OSA from prior audit and had not had any updates since 2011 NPIP Audit.

	SOP	Version Date	Version
S	M. Gallisepticum ELISA	4/13/09	2.0
S	M. Synoviae ELISA	4/13/09	2.0
S	Mycoplasma Gallisepticum-Synoviae ELISA	6/28/2010	Original
S	Pullorum Agglutination Test	4/13/09	2.0
M	Mycoplasma Synoviae PCR	10/20/2010	2.0
M	Mycoplasma Gallisepticum PCR	10/20/2010	2.0
M	Salmonella Enteritidis PCR	1/17/2011	1.0
M	Avian Influenza Virus Type A Antigen Test	4/7/2010	1.0
M	RNA Extraction using MagMAX on BioSprint 96	5/5/2009	2.0
M	Gel Electrophoresis	10/6/2010	2.0
M	Lysis of Gram Negative Cultures	2/20/2009	1.0
M	Extraction of RNA using Ambion Mag MAXtm AI/ND viral RNA Isolation Kit	3/17/2010	4.0
M	Extraction of AIV/NDV Using the Qiagen RNeasy Method	11/11/2008	2.1
M	Extraction of AIV/NDV from Tissues	11/12/2008	1.0
M	AIV Matrix Real Time RT-PCR (Qiagen Chemistry)	2/5/2009	2.1

(3) Laboratory Protocol

- SOP review -- NC example

Requirement	Findings
Standard Operating Procedures ▪ Written instructions for all testing activities ▪ Properly referenced (e.g. NPIP, NVSL) ▪ Document control to ensure current version of SOP is available	▪ Electronic copies of standard operating procedures were provided for NPIP testing activities: appropriate references were included. ▪ The *Mycoplasma* SOP still did not clearly specify the methods used to re-test positive samples based upon NPIP recommendations (previous finding in 2010 audit) ▪ Procedures and work instructions were uniquely labeled as evidence of document control.

(3) Laboratory Protocol

- SOP review -- SC example

Written SOPs for all Testing Activities	SOP Available	Proper Reference and Document Controls	Remarks
Pullorum-Typhoid	X yes □ no	X yes □ no	
S. Enteritidis	X yes □ no	X yes □ no	
Avian Influenza	X yes □ no	X yes □ no	Follow NAHLN (AVPRO1510)
Mycoplasma Synoviae	X yes □ no	X yes □ no	
Mycoplasma Gallisepticum	X yes □ no	X yes □ no	
Salmonella	X yes □ no	X yes □ no	
Other_____	□ yes □ no	□ yes □ no	

(3) Laboratory Protocol

- Suggestions for this section:
  - Equipment
    - Suitable for test activities
    - Maintenance program with records
    - Calibration program with records (balances, ELISA readers, centrifuges, micropipettes, thermometers)
    - Uniquely identified
    - Monitoring records for temperature dependent activities (e.g. incubators)

(3) Laboratory Protocol

- Equipment review – NC example

Equipment ▪ Suitable for test activities ▪ Maintenance program with records ▪ Calibration program with records (balances, ELISA readers, centrifuges, micropipettes, thermometers) ▪ Uniquely identified ▪ Monitoring records for temperature dependent activities e.g. incubators	▪ Laboratory equipment used for NPIP testing activities was in good repair and suitable for the tests. ▪ No maintenance program was in place for relevant equipment. ▪ Temperature monitoring was being conducted for incubators, freezers and refrigerators. Current calibration certificates for thermometers was not available. ▪ Vendors used to calibrate pipettors and scales were ISO certified. ▪ Equipment was uniquely identified. ▪ Spot checks of pipettors, ELISA reader, and thermometers revealed current calibration status.

(3) Laboratory Protocol

- Suggestions for this section:
  - Accommodation and environmental conditions
    - Suitable environment for conducting tests
    - Monitor, control and record environmental conditions, as they relate to conducting lab tests (e.g. monitor ambient temperatures for ELISAs)

(3) Laboratory Protocol

- Accommodation and environmental conditions review – KY example

	the process of calibrating all pipettors in each specific lab.
Accommodation and environmental conditions • Suitable environment for conducting tests • Monitor, control and record environmental conditions, as they relate to conducting lab tests e.g. monitor ambient temp. for ELISAs • Safety, biosafety and biosecurity	• Environment appeared to be suitable for test. The laboratory was clean, neat and very organized. • The temperature charts for the serology rooms were reviewed and within appropriate temperature for conducting ELISA test. • Laboratory has safety, biosafety and biosecurity policy and training requirements for employees are available.

(3) Laboratory Protocol

- Suggestions for this section:
  - Quality Control
    - Use of appropriate test controls with records
    - Reagent / media QC records
    - Use of test kits, antisera, reagents and media that have not surpassed their expiration dates

(3) Laboratory Protocol

- Quality Control Review – NC, SC example

Quality Control ▪ Use of appropriate test controls with records ▪ Reagent/ media QC records ▪ Use of test kits, antisera, reagents and media that have not surpassed their expiration dates	▪ QC records for MS plate test were reviewed and these included date, initials of technician, lot number, expiration date, results and pass/fail status of the controls. ▪ AI test controls and *Mycoplasma* plate antigen being used were well within their expiration date. ▪ ELISA test kits that were in use were also observed to be well-within their expiry date and were properly stored. ▪ No QC records could be produced for Salmonella antisera

Quality Control	Lab Section	Yes	No	Remarks
Use of appropriate test controls with records	Microbiology Molecular Serology	□ X X	□ □ □	N/A for micro AI per batch (NVSL), MS, MG, PT per test (NVSL)
Reagent/media QC records	Microbiology Molecular Serology	X X X	□ □ □	 AI AGID media QC per batch
Use of test kits, antisera, reagents and media that have not surpassed their expiration dates	Microbiology Molecular Serology	X X X	□ □ □	Serogroup, VITEK, BBL Crystals, plate Primary stock solutions Test kits

(3) Laboratory Protocol

- Suggestions for this section:
  - Specimens
    - Uniquely identified
    - Methods to ensure specimens are appropriate and of suitable quality for test
    - Proper storage and preservation

(3) Laboratory Protocol

- Specimens – KY, SC example

Specimens ▪ Uniquely identified ▪ Methods to ensure specimens are appropriate and of suitable quality for test ▪ Proper storage and preservation	▪ Specimens were uniquely identified. Recently submitted samples within the serology lab indicated identification that corresponded with the accession number assigned when specimens enter the lab. ▪ The laboratory will not test specimens that are not appropriate and not suitable for testing. Sera was observed as being of good quality and a few examples of poor quality. Laboratory communicates with the NPIP office and company who is submitting samples if poor in quality. These samples are not tested and are reported that they are not suitable for test on the lab submission form and NPIP VS 9-2 report. ▪ The specimens are stored and preserved appropriately. Numerous sample sets were observed as being stored under refrigeration.

Specimens	Lab Section	Yes	No	Remarks
Uniquely identified	Microbiology Molecular Serology	X X X	□ □ □	
Methods to ensure specimens are appropriate and of suitable quality for test	Microbiology Molecular Serology	X X X	□ □ □	
Proper storage and preservation	Microbiology Molecular Serology	X X X	□ □ □	

(3) Laboratory Protocol

- Suggestions for this section:
  - Reporting Test Results
    - Employee performing test initials and dates worksheets or reports
    - Supervisory review of client reports
    - Test report includes: title, lab name and address, unique identification, name of client, date of receipt and report date

(3) Laboratory Protocol

- Reporting Test Results – KY, SC example

Reporting Test Results ▪ Employee performing test initials and dates worksheets or reports ▪ Supervisory review of client reports ▪ Test report includes: title, lab name and address, unique identification, name of client, date of receipt and report date ▪ Preliminary or Final Reports distinguished ▪ Corrections identified as such ▪ Test turnaround time ▪ MOU	▪ Worksheets that were reviewed indicated the technician running the test. ▪ There is supervisory review of all client reports The test report includes the appropriate information, such as the flock identification, house number, number of birds and collection date. ▪ There are preliminary reports when needed before the final report. ▪ Amended reports are indicated if there have been any corrections to the reports. ▪ Turnaround time is appropriate per the test requested and performed. ▪ 2 MOU's are currently in place between the laboratory and the KPF-KY OSA of NPIP.

Reporting Test Results	Lab Section	Yes	No	Remarks
Employee performing test initials and dates worksheets or reports	Microbiology Molecular Serology	X X X	□ □ □	
Test report includes: title, lab name and address, unique identification, name of client, date of receipt and report date	Microbiology Molecular Serology	X X X	□ □ □	Use USAHERDS
Preliminary or Final Reports distinguished	Microbiology Molecular Serology	X X X	□ □ □	
Corrections identified as such	Microbiology Molecular Serology	X X X	□ □ □	
Test turnaround time	Microbiology Molecular Serology	X X X	□ □ □	

(4) State Site Visit

- The Official State Agency (OSA) will conduct a site visit and recordkeeping audit annually.

(5) Service Review

- Authorized laboratories will be reviewed by the Service (NPIP staff) every 3 years.
- The Service review may include, but will not necessarily be limited to, checking records, laboratory protocol, check-test proficiency, technician training, and peer review.

(6) Reporting

- A MOU or other means shall be used to establish testing and reporting criteria,
  - including criteria that provide for reporting H5 and H7 LPAI directly to the Service (NVSL)

- *Salmonella pullorum* and *Mycoplasma* Plan disease reactors must be reported to the OSA within 48 hours

(6) Reporting

- A MOU (or other document) consists of:
  - State the parties involved: OSA and Lab
  - Purpose of MOU
  - What the Authorized Laboratory agrees to do
  - What the OSA agrees to do
  - Reporting criteria
  - Signature lines
  - MOU effective until …..

(7) Verification

- Random samples may be required to be submitted for verification as specified by the OSA.


THANKS